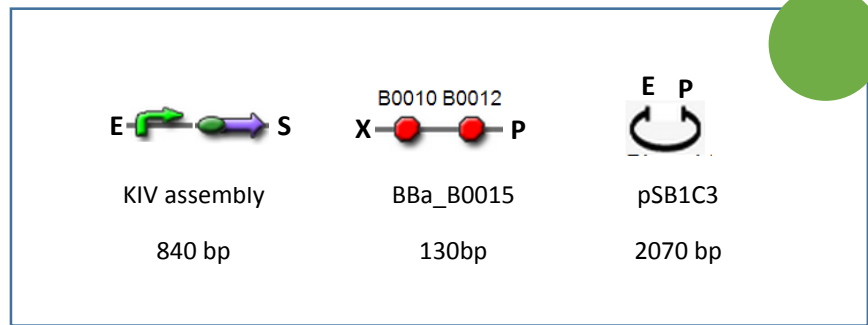


## Assembly:



## 1<sup>st</sup> Day

### EXSP Digestion (see **Enzymatic Digestion Protocol**)

Parts	ng/ul	Volume to 2,5 ug (ul)	Buffer x10 (ul)	EcoRI (ul)	XbaI (ul)	SpeI (ul)	PstI (ul)	H <sub>2</sub> O to 50ul (ul)
KIV assembly	272,0	9,5	5	1	-	1	-	33,5
BBa_B0015	118	3,0 ug = 26 ul	5	-	1	-	1	17
pSB1C3	288,0	3,5 ug = 12 ul	5	1	-	-	1	31

Repeat this digestion only if you run out of stock

## 2<sup>nd</sup> Day

### Gel Purification

- See **Kit Wizard SV gel and PCR clean up Promega Protocol**
- Quantify digestion products

Parts	ng/ul	260/280
KIV assembly (ES)	14,2	1,89
BBa_B0015 (XP)	6,0	1,79
pSB1C3 (EP)	31,0	2,05

**Obs:** 260/280 is a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

### Ligation (see **Ligation Protocol**)

Linear Plasmid 50 ng	2 ul	
Insert : Plasmid 3:1 (KIV) ; 6:1 (BBa_B0015)	KIV	BBa_B0015
	4,5 ul	4 ul
5x T4 DNA Buffer	4 ul	
T4 DNA ligase 1-5 u	0,5 ul	
H <sub>2</sub> O to 20 ul	5 ul	

**Obs:** To determinate the amount of DNA necessary we used the following equation

$$\text{Insert ng} = \text{plasmid ng} \times \frac{\text{insert bp}}{\text{plasmid bp}} \times \text{insert:plasmid ratio}$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

### 3<sup>rd</sup> Day

Transformation (see **Transformation Protocol in *Escherichia coli* DH5-α**)

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Organism: *E. coli* DH5-α

Selection: Cloranphenicol

### 4<sup>th</sup> Day

- Inoculate 3 – 4 colonies overnight in a 6 ml LB with the same antibiotic used in the transformation protocol.
- Incubate overnight at 275rpm/37°C.

### 5<sup>th</sup> Day

Miniprep

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- Prepare **glycerol stock** of the clones (500ul glycerol 40% + 500ul inoculum).
- Extract plasmidial DNA (see **Alkaline Lyses or PureLink Invitrogen Protocol**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

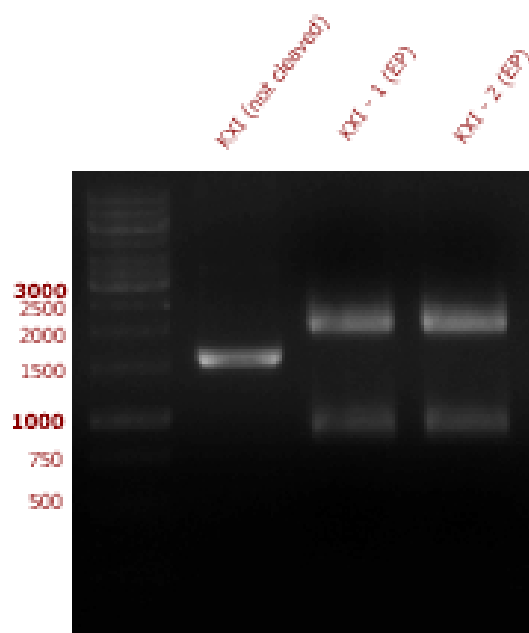
Assembly Confirmation

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- EP Digestion (see **Enzymatic Digestion Protocol**)

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	EcoRI (ul)	PstI (ul)	H <sub>2</sub> O to 10ul (ul)
KXI – 1	2	1	0,5	0,5	6
KXI – 2	2	1	0,5	0,5	6

- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the EP digestion



Size expected	Size in gel
970 bp	~ 950 bp